09/760,085

```
> s (chelat? or EDTA)(10a)(divalent or magnesium)(10A)(chaotrop? or guanidinium
isothiocyanate)
L1            6 (CHELAT? OR EDTA)(10A)(DIVALENT OR MAGNESIUM)(10A)(CHAOTROP? OR
                GUANIDINIUM ISOTHIOCYANATE)

=> s l1 and ((purif? or isolat? or extract?)(10a)(DNA or RNA or nucleic acid#))
   1 FILES SEARCHED...
L2            5 L1 AND ((PURIF? OR ISOLAT? OR EXTRACT?)(10A)(DNA OR RNA OR NUCLE
                IC ACID#))

=>

=>

=>

=> s l2 and single strand##
L3            4 L2 AND SINGLE STRAND##

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4             4 DUP REM L3 (0 DUPLICATES REMOVED)

=> d l4 1-4 bib ab kwic

L4   ANSWER 1 OF 4  USPATFULL
AN     1999:117261  USPATFULL
TI     Detection of toxigenic marine diatoms of the genus Pseudo-nitzschia
IN     Scholin, Christopher A., Monterey, CA, United States
       Cangelosi, Gerard A., Seattle, WA, United States
       Haydock, Paul V., Seattle, WA, United States
PA     Monterey Bay Aquarium Research Institute, Moss Landing, CA, United
       States (U.S. corporation)
PI     US 5958689              19990928
AI     US 1997-861096          19970521 (8)
PRAI   US 1996-18143P      19960522 (60)
DT     Utility
FS     Granted
EXNAM  Primary Examiner: Jones, W. Gary; Assistant Examiner: Whisenant, Ethan
LREP   Townsend and Townsend and Crew LLP
CLMN   Number of Claims: 23
ECL    Exemplary Claim: 11
DRWN   No Drawings
LN.CNT 1893
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention provides compositions, methods, and kits for
       detecting species of Pseudo-nitzschia from a marine sample.
       Oligonucleotide probes for rRNA hypervariable regions of the
       Psuedo-nitzschia species: P. australis, P. pungens, P. multiseries, P.
       pseudodelicatissima, P. heimii, P. fraudulenta, P. delicatissima, and P.
       americana are provided as well as a oligonucleotide probe for a
       conserved region of ribosomal RNA from Pseudo-nitzschia.
DETD   The terms "oligonucleotide" or "polynucleotide" probes are meant to
       include both double stranded and single stranded DNA
       or RNA. The terms also refer to synthetically or recombinantly derived
       sequences essentially free of non-nucleic acid contamination.
DETD   . . . of nucleotides having said hybridization capability. The probe
       can be free or contained within a vector sequence (e.g., plasmids or
       Single Stranded DNA).
DETD   . . . RNA into a replication vector, such as pBR322, M13, or into a
       vector containing the SP6 promotor (e.g., generation of single
       -stranded RNA using SP6 RNA polymerase), and transformation of
       a bacterial host. The DNA probes can be purified
       from the host cell by lysis and nucleic acid
```

extraction, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis. The use of polymerase chain reaction technology can also be used to obtain large quantities of probe.. . .

DETD . . . reference. Lysing solutions are well known in the art and are typically composed of a buffered detergent solution having a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). Generally, these buffers. . .

DETD . . . a hybridization solution for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

L4 ANSWER 2 OF 4 USPATFULL
AN 95:1508 USPATFULL
TI Oligonucleotide probes for detection of periodontal pathogens
IN Schwartz, Dennis E., Redmond, WA, United States
Kanemoto, Roy H., Seattle, WA, United States
Watanabe, Susan M., Seattle, WA, United States
Dix, Kim, Arlington, WA, United States
PA MicroProbe Corporation, Bothell, WA, United States (U.S. corporation)
PI US 5378604 19950103
AI US 1993-3367 19930112 (8)
DCD 20190518
RLI Continuation of Ser. No. US 1990-571563, filed on 29 Aug 1990, now patented, Pat. No. US 5212059 And a continuation-in-part of Ser. No. US 1988-142106, filed on 11 Jan 1988, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Parr, Margaret; Assistant Examiner: Escallon, Miguel H.
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1359
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to compositions of oligonucleotide probes for use in the detection of bacteria associated with medical disorders of the human mouth, wherein said probes consist essentially of a segment of nucleic acid capable of selectively hybridizing under hybridizing conditions, to the 16S or 23S ribosomal RNA [rRNA] of said bacteria. Methods for detection, as well as diagnostic kits for the assay of these bacterium, are also disclosed.

SUMM . . . of nucleotides having said hybridization capability- The probe can be free or contained within a vector sequence (e.g., plasmids or **Single Stranded** DNA).

SUMM The terms oligonucleotide or polynucleotide probes are meant to include both double stranded and **single stranded** DNA or RNA. The terms also refer to synthetically or recombinantly derived sequences essentially free of non-nucleic acid contamination.

SUMM . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.

SUMM . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts

with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides, dNTP, by. . .

SUMM . . . a moderate temperature for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

SUMM . . . containing oral pathogenic bacteria are first subjected to a lysing solution, such as a buffered solution of detergent and a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent, a reducing agent and a **divalent** metal **chelator**. The sample may be directly fixed to a support or further processed to **extract nucleic acids**. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like.

DETD Oligonucleotides were synthesized on an Applied Biosystems **DNA** synthesizer Model 380B via .beta.-cyanoethylphosphoramidite chemistry. The oligonucleotides were **purified** by preparative polyacrylamide gel electrophoresis or by high pressure liquid chromatography and eluted as detailed in Applied Biosystems User Bulletin. . .

DETD E. **Isolation** of **Nucleic Acid** from Bacterial Culture or Subgingival plaque Samples

DETD The **extracted** microbial **nucleic acids** are immobilized onto Nytran as follows: 380 .mu.l of nucleic acid (at less than 6 .mu.g/380 .mu.l) in TE and. . .

DETD . . . probes are synthesized with an ethylamine group at the 5' end, biotinylated with NHS-LC-biotin (Pierce Chemical Co., Rockford, Ill.), and **purified** by Elutip-D chromatography. **Nucleic acids**, immobilized on Nytran or nitrocellulose membranes, are hybridized with 5 ng/ml of oligonucleotide probe in 0.6M NaCl, 90 mM Tris-HCl. . .

DETD . . . the ribosomal RNA with minimal secondary and tertiary interactions are defined by solution hybridization and sandwich assay methods. For example, **purified** ribosomal **RNA** (1-5 ug) was hybridized with .sup.32 P-labelled complementary oligonucleotide probes (5-10 ng), such as those in Table 1 or Table. . .

L4 ANSWER 3 OF 4 USPATFULL

AN 94:66394 USPATFULL

TI Quantification of bacteria using a nucleic acid hybridization assay

IN Adams, Trevor H., Woodinville, WA, United States
Schwartz, Dennis E., Redmond, WA, United States
Vermuelen, Nicolaas M. J., Woodinville, WA, United States
Kanemoto, Roy H., Seattle, WA, United States

PA Microprobe Corporation, Bothell, WA, United States (U.S. corporation)

PI US 5334501 19940802

AI US 1993-41804 19930401 (8)

RLI Continuation of Ser. No. US 1990-631131, filed on 19 Dec 1990, now abandoned which is a continuation of Ser. No. US 1989-378355, filed on 11 Jul 1989, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Fleisher, Mindy B.

LREP Townsend and Townsend Khourie and Crew

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 1008

AB This invention provides for a method of quantifying bacteria using a bacterial specific nucleic acid probe which is complementary to a unique

and highly conserved region of the 16S ribosomal RNA (rRNA) of bacteria. This probe permits the rapid detection of 16S rRNA in a sample and by comparison with known standards, one can estimate the total bacterial count in the sample. The method is accurate and reproducible and conducted at temperatures of between about 120.degree. to about 40.degree. C.

SUMM The term "lysate" refers to solutions containing bacterial **nucleic acid**. A lysate would include crude mixtures of disrupted bacteria, semi-**purified** solutions and **purified** solutions of bacterial **nucleic acid**

.

DETD . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.

DETD . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides (dNTP), by. . .

DETD . . . and 8.0, and contain both chelating agents and surfactants. Typically, a lysing solution is a buffered detergent solution having a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). The use of. . .

DETD The sample may be directly immobilized to a support or further processed to **extract nucleic acids** prior to immobilization. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like. The immobilized nucleic acid. . .

DETD . . . a moderate temperature for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

DETD . . . signal strength of unknowns with that of the standards. It has previously been shown on Nytran slot blots with total **nucleic acid extracts** of a panel of 72 strains of 14 different bacteria that the signal strengths were comparable when hybridized with .sup.32. . .

DETD . . . live bacteria it is expected that probe cell count will generally be higher, since it detects the presence of total **nucleic acid isolated** from both viable and non-viable bacteria.

DETD . . . for several days. Upon thawing, the samples were treated with 1% W/V SDS and 1 mg/ml proteinase K. The total **nucleic acid** was **extracted** with two phenol-chloroform **extractions** and then precipitated with ethanol. The pellet was resuspended in TE (10 mM Tris, 1MM EDTA), heated for one minute. . .

DETD The total **nucleic acid** from a known number of actively growing cultured bacteria were **extracted** as above, then **nucleic acid** carefully **extracted**, serially diluted, slotted and subsequently probed with the same universal primer oligonucleotide. The resulting autoradiograph indicated the intensity of the. . .

DETD . . . bacteria including the following genera: Actinobacillus, Haemophilus, Bacteroides, Eikenella, Fusobacterium, Wolinella, Campylobacter, Escherichia, Peptostreptococcus, Streptococcus, Capnocytophaga, Selenomonas, Actinomyces and Fusobacterium.

Nucleic acids from the different bacteria were **extracted** and slotted onto a Nytran filter. This filter was then probed with a kinased UP9A oligo in a 30% formamide,. . .

L4 ANSWER 4 OF 4 USPATFULL
AN 93:39885 USPATFULL
TI Oligonucleotide probes for the detection of periodontal pathogens
IN Schwartz, Dennis E., Redmond, WA, United States
Kanemoto, Roy H., Seattle, WA, United States
Watanabe, Susan M., Seattle, WA, United States
Dix, Kim, Arlington, WA, United States
PA MicroProbe Corporation, Bothel, WA, United States (U.S. corporation)
PI US 5212059 19930518
AI US 1990-571563 19900829 (7)
WO 1989-US72 19890109
19900829 PCT 371 date
19900829 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1988-142106, filed on 11 Jan 1988, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Escallon, M.
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1519
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to compositions of oligonucleotide probes for use in the detection of bacteria associated with medical disorders of the human mouth, wherein said probes consist essentially of a segment of nucleic acid capable of selectively hybridizing under hybridizing conditions, to the 16S or 23S ribosomal RNA [rRNA] of said bacteria. Methods for detection, as well as diagnostic kits for the assay of these bacterium, are also disclosed.
SUMM . . . of nucleotides having said hybridization capability. The probe can be free or contained within a vector sequence (e.g., plasmids or **Single Stranded** DNA).
SUMM The terms oligonucleotide or polynucleotide probes are meant to include both double stranded and **single stranded** DNA or RNA.
The terms also refer to synthetically or recombinantly derived sequences essentially free of non-nucleic acid contamination.
DETD . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.
DETD . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides, dNTP, by. . .
DETD . . . a moderate temperature for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .
DETD . . . containing oral pathogenic bacteria are first subjected to a lysing solution, such as a buffered solution of detergent and a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent, a reducing agent and a **divalent** metal **chelator**. The sample may

be directly fixed to a support or further processed to **extract** **nucleic acids**. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like.

DETD Oligonucleotides were synthesized on an Applied Biosystems **DNA** synthesizer Model 380B via .beta.-cyanoethylphosphoramidite chemistry. The oligonucleotides were **purified** by preparative polyacrylamide gel electrophoresis or by high pressure liquid chromatography and eluted as detailed in Applied Biosystems User Bulletin. . .

DETD E. **Isolation** of **Nucleic Acid** from Bacterial Culture or Subgingival Plaque Samples

DETD The **extracted** microbial **nucleic acids** are immobilized onto Nytran as follows: 380 .mu.l of nucleic acid (at less than 6 .mu.g/380 .mu.l) in TE. . .

DETD . . . probes are synthesized with an ethylamine group at the 5' end, biotinylated with NHS-LC-biotin (Pierce Chemical Co., Rockford, Ill.), and **purified** by Elutip-D chromatography. **Nucleic** **acids**, immobilized on Nytran or nitrocellulose membranes, are hybridized with 5 ng/ml of oligonucleotide probe in 0.6M NaCl, 90 mM Tris-HCl. . .

DETD . . . the ribosomal RNA with minimal secondary and tertiary interactions are defined by solution hybridization and sandwich assay methods. For example, **purified** ribosomal **RNA** (1-5 ug) was hybridized with .sup.32 P-labelled complementary oligonucleotide probes (5-10 ng), such as those in Table 1 or Table. . .

=> d l2 1-5 bib ab

L2 ANSWER 1 OF 5 USPATFULL
AN 2002:157007 USPATFULL
TI Methods for detecting and identifying a gram positive bacteria in a sample
IN Trieu-Cuot, Patrick, Fortenay aux Roses, FRANCE
Poyart, Clare, Fortenay aux Roses, FRANCE
PA INSTITUT PASTEUR, Paris Cedex, FRANCE, 75724 (non-U.S. corporation)
PI US 2002081606 A1 20020627
AI US 2001-860432 A1 20010521 (9)
PRAI US 2000-205237P 20000519 (60)
DT Utility
FS APPLICATION
LREP OBLON SPIVAK MCCLELLAND MAIER & NEUSTADT PC, FOURTH FLOOR, 1755 JEFFERSON DAVIS HIGHWAY, ARLINGTON, VA, 22202
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 1670
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides fragments of a sodA gene from gram positive bacteria, methods of using these fragments as probes to detect and identify microorganisms in a sample and kits containing suitable reagents to perform the method.

L2 ANSWER 2 OF 5 USPATFULL
AN 1999:117261 USPATFULL
TI Detection of toxigenic marine diatoms of the genus Pseudo-nitzschia
IN Scholin, Christopher A., Monterey, CA, United States
Cangelosi, Gerard A., Seattle, WA, United States
Haydock, Paul V., Seattle, WA, United States
PA Monterey Bay Aquarium Research Institute, Moss Landing, CA, United States (U.S. corporation)

PI US 5958689 19990928
AI US 1997-861096 19970521 (8)
PRAI US 1996-18143P 19960522 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Whisenant, Ethan
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 23
ECL Exemplary Claim: 11
DRWN No Drawings
LN.CNT 1893
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides compositions, methods, and kits for detecting species of Pseudo-nitzschia from a marine sample. Oligonucleotide probes for rRNA hypervariable regions of the Psuedo-nitzschia species: P. australis, P. pungens, P. multiseries, P. pseudodelicatissima, P. heimii, P. fraudulenta, P. delicatissima, and P. americana are provided as well as a oligonucleotide probe for a conserved region of ribosomal RNA from Pseudo-nitzschia.

L2 ANSWER 3 OF 5 USPATFULL
AN 95:1508 USPATFULL
TI Oligonucleotide probes for detection of periodontal pathogens
IN Schwartz, Dennis E., Redmond, WA, United States
Kanemoto, Roy H., Seattle, WA, United States
Watanabe, Susan M., Seattle, WA, United States
Dix, Kim, Arlington, WA, United States
PA MicroProbe Corporation, Bothell, WA, United States (U.S. corporation)
PI US 5378604 19950103
AI US 1993-3367 19930112 (8)
DCD 20190518
RLI Continuation of Ser. No. US 1990-571563, filed on 29 Aug 1990, now patented, Pat. No. US 5212059 And a continuation-in-part of Ser. No. US 1988-142106, filed on 11 Jan 1988, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Parr, Margaret; Assistant Examiner: Escallon, Miguel H.
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1359
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to compositions of oligonucleotide probes for use in the detection of bacteria associated with medical disorders of the human mouth, wherein said probes consist essentially of a segment of nucleic acid capable of selectively hybridizing under hybridizing conditions, to the 16S or 23S ribosomal RNA [rRNA] of said bacteria. Methods for detection, as well as diagnostic kits for the assay of these bacterium, are also disclosed.

L2 ANSWER 4 OF 5 USPATFULL
AN 94:66394 USPATFULL
TI Quantification of bacteria using a nucleic acid hybridization assay
IN Adams, Trevor H., Woodinville, WA, United States
Schwartz, Dennis E., Redmond, WA, United States
Vermuelen, Nicolaas M. J., Woodinville, WA, United States
Kanemoto, Roy H., Seattle, WA, United States
PA Microprobe Corporation, Bothell, WA, United States (U.S. corporation)
PI US 5334501 19940802
AI US 1993-41804 19930401 (8)
RLI Continuation of Ser. No. US 1990-631131, filed on 19 Dec 1990, now abandoned which is a continuation of Ser. No. US 1989-378355, filed on

11 Jul 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Fleisher, Mindy B.
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1008
AB This invention provides for a method of quantifying bacteria using a bacterial specific nucleic acid probe which is complementary to a unique and highly conserved region of the 16S ribosomal RNA (rRNA) of bacteria. This probe permits the rapid detection of 16S rRNA in a sample and by comparison with known standards, one can estimate the total bacterial count in the sample. The method is accurate and reproducible and conducted at temperatures of between about 120.degree. to about 40.degree. C.

L2 ANSWER 5 OF 5 USPATFULL
AN 93:39885 USPATFULL
TI Oligonucleotide probes for the detection of periodontal pathogens
IN Schwartz, Dennis E., Redmond, WA, United States
Kanemoto, Roy H., Seattle, WA, United States
Watanabe, Susan M., Seattle, WA, United States
Dix, Kim, Arlington, WA, United States
PA MicroProbe Corporation, Bothel, WA, United States (U.S. corporation)
PI US 5212059 19930518
AI US 1990-571563 19900829 (7)
WO 1989-US72 19890109
19900829 PCT 371 date
19900829 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1988-142106, filed on 11 Jan 1988, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Escallon, M.
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1519
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to compositions of oligonucleotide probes for use in the detection of bacteria associated with medical disorders of the human mouth, wherein said probes consist essentially of a segment of nucleic acid capable of selectively hybridizing under hybridizing conditions, to the 16S or 23S ribosomal RNA [rRNA] of said bacteria. Methods for detection, as well as diagnostic kits for the assay of these bacterium, are also disclosed.

=> d l2 1 kwic

L2 ANSWER 1 OF 5 USPATFULL
DETD . . . and 8.0, and contain both chelating agents and surfactants. Typically, a lysing solution is a buffered detergent solution having a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). The use of. . .
DETD [0009] The sample may be directly immobilized to a support or further processed to **extract nucleic acids** prior to immobilization. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon,

nitrocellulose, diazobenzyloxymethyl cellulose, and the like. The immobilized nucleic acid. . .

DETD . . . of the bacterial strains used in this study, including the type strains, are listed in Table 1 and 2. Rapid **extraction** of bacterial genomic **DNA** was carried out by using the InstaGene.TM. Matrix (Bio-Rad, Hercules, Calif.) on cells collected from 2 ml of an overnight. . .

=> s l4 and (magnesium or Mg)
L5 4 L4 AND (MAGNESIUM OR MG)

=> d l5 1-4 kwic

L5 ANSWER 1 OF 4 USPATFULL

DETD The terms "oligonucleotide" or "polynucleotide" probes are meant to include both double stranded and **single stranded** DNA or RNA. The terms also refer to synthetically or recombinantly derived sequences essentially free of non-nucleic acid contamination.

DETD . . . of nucleotides having said hybridization capability. The probe can be free or contained within a vector sequence (e.g., plasmids or **Single Stranded** DNA).

DETD . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis. The use of polymerase chain reaction technology can also be used to obtain large quantities of probe.. . .

DETD . . . reference. Lysing solutions are well known in the art and are typically composed of a buffered detergent solution having a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). Generally, these buffers. . .

DETD . . . serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 **mg**/ml, fragmented nucleic DNA, e.g., calf thymus or salmon sperm DNA, or yeast RNA, and optionally from about 0.5 to 2%. . .

DETD . . . a hybridization solution for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

DETD . . . rinsed briefly in 500-750 ml freshly prepared hybridization buffer [5.times.SET (1/5 dilution of above), 1% (v/v) Nonidet P-40 (Sigma), 12.5 **mg**/ml polyadenlyic acid (poly A; Sigma)]. Cells were pelleted as before, then resuspended in 150 ml of hybridization buffer. Approximately 47.5. . .

DETD . . . cells were rinsed briefly in 500-750 .mu.L freshly prepared hybridization buffer [5.times.-7.times.SET (see Table 3), 0.1% (v/v) Nonidet P-40, 25 **mg** mL.sup.-1 polyadenylic acid (poly A)]. Cells were pelleted as before, then resuspended in 150 .mu.L of hybridization buffer. Approximately 47.5. . .

DETD . . . several minutes at room temperature. Cells were collected again by vacuum filtration, and 0.5 ml of hybridization buffer containing 5 **mg** of fluorescein-labeled aus D1 probe were added. Filter stacks were capped and the entire filtration manifold was immersed in a. . .

L5 ANSWER 2 OF 4 USPATFULL

SUMM . . . of nucleotides having said hybridization capability- The probe can be free or contained within a vector sequence (e.g., plasmids or

Single Stranded DNA).

SUMM The terms oligonucleotide or polynucleotide probes are meant to include both double stranded and **single stranded** DNA or RNA. The terms also refer to synthetically or recombinantly derived sequences essentially free of non-nucleic acid contamination.

SUMM . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.

SUMM . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides, dNTP, by. . .

SUMM . . . serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 **mg**/ml, fragmented nucleic DNA, e.g., calf thymus or salmon sperm DNA, or yeast RNA, and optionally from about 0.5 to 2%. . .

SUMM . . . a moderate temperature for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

SUMM . . . containing oral pathogenic bacteria are first subjected to a lysing solution, such as a buffered solution of detergent and a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent, a reducing agent and a **divalent** metal **chelator**. The sample may be directly fixed to a support or further processed to **extract nucleic acids**. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like.

DETD Bacterial cells are resuspended in a lysis solution (20 **mg**/ml lysozyme, 25% sucrose, 50 mM Tris, pH 8, 10 mM EDTA), and incubated at 37.degree. C. for 30 min. Sodium dodecylsulfate (1-2% w/v) and pronase E (1 **mg**/ml) or proteinase K (200 .mu.g/ml) are added, and the solution is incubated 30 min at 37.degree. C. The lysates are. . . v/v) and then precipitated with ethanol. Nucleic acid is pelleted, washed with 70% v/v ethanol, and resuspended to approximately 1 **mg**/ml in 1.times. TE buffer (10 mM Tris, pH 8, 1 mM EDTA). Resuspended nucleic acid is stored at -70.degree. C.

DETD . . . up as follows. Two .mu.l of primer are added to 3 .mu.l of a solution of bacterial nucleic acid (0.5-25 **mg**/ml), 2 .mu.l 5.times. HYB buffer (500 mM KCl, 250 mM Tris-HCl, pH 8.5), and 3 .mu.l of H.sub.2 O. The. . .

DETD Oligonucleotides were synthesized on an Applied Biosystems **DNA** synthesizer Model 380B via .beta.-cyanoethylphosphoramidite chemistry. The oligonucleotides were **purified** by preparative polyacrylamide gel electrophoresis or by high pressure liquid chromatography and eluted as detailed in Applied Biosystems User Bulletin. . .

DETD E. **Isolation** of **Nucleic Acid** from Bacterial Culture or Subgingival plaque Samples

DETD . . . 50 mM Tris-HCl (pH 8.0), is added to the sample and vortexed briefly. 50 .mu.l of freshly made lysozyme (10 **mg**/ml in 0.25.times. bacterial sucrose lysis buffer; Sigma Chemical), is added and the sample incubated for 15 min at 37.degree. C. 75 .mu.l of 10% SDS is then added and the sample vortexed briefly. 75 .mu.l of Pronase E (10 **mg**/ml, Sigma Chemical; self-digested as per Maniatis, et al.,

Molecular Cloning: A Laboratory Manual) is added, the sample vortexed briefly and. . .

DETD The **extracted** microbial **nucleic acids** are immobilized onto Nytran as follows: 380 .mu.l of nucleic acid (at less than 6 .mu.g/380 .mu.l) in TE and. . .

DETD . . . probes are synthesized with an ethylamine group at the 5' end, biotinylated with NHS-LC-biotin (Pierce Chemical Co., Rockford, Ill.), and **purified** by Elutip-D chromatography. **Nucleic acids**, immobilized on Nytran or nitrocellulose membranes, are hybridized with 5 ng/ml of oligonucleotide probe in 0.6M NaCl, 90 mM Tris-HCl. . .

DETD . . . the ribosomal RNA with minimal secondary and tertiary interactions are defined by solution hybridization and sandwich assay methods. For example, **purified** ribosomal **RNA** (1-5 ug) was hybridized with .sup.32 P-labelled complementary oligonucleotide probes (5-10 ng), such as those in Table 1 or Table. . .

DETD . . . is derivatized with the thiol-reactive agent N-succinimidyl(4-iodoacetyl)aminobenzoate ("SIAB") through the amino linker arm. The SIAB-oligonucleotide is prepared by adding 1.2 **mg** SIAB to 300 .mu.g of the oligonucleotide, incubating for one hour at room temperature, and desalting over a G-25 column. . .

DETD Alkaline phosphatase is thiolated with dithiobis(succinimidylpropionate) ("DSP") by adding 800 .mu.g DSP to 4 **mg** alkaline phosphatase. The reaction is allowed to proceed for 30 min at room temperature. The reaction mixture then is treated. . .

DETD . . . room temperature the IO.sub.4 is removed by gel filtration over Sephadex G-25, and the material concentrated to less than 20 **mg** /ml in 1 mM NaOAc (pH 4.5). The concentrated hRP is then used to resuspend a pellet of oligonucleotide with a. . .

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SUMM The term "lysate" refers to solutions containing bacterial **nucleic acid**. A lysate would include crude mixtures of disrupted bacteria, semi-**purified** solutions and **purified** solutions of bacterial **nucleic acid** .

DETD . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.

DETD . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides (dNTP), by. . .

DETD . . . and 8.0, and contain both chelating agents and surfactants. Typically, a lysing solution is a buffered detergent solution having a **divalent** metal **chelator** or a buffered **chaotrophic** salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). The use of. . .

DETD The sample may be directly immobilized to a support or further processed to **extract nucleic acids** prior to immobilization. Released or **extracted** bacterial **nucleic acid** (including target **nucleic acid**) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like. The immobilized nucleic acid. . .

DETD . . . serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 **mg**/ml, fragmented nucleic DNA, e.g., calf thymus or salmon sperm DNA, or yeast RNA, and optionally from about 0.5 to 2%. . .

DETD . . . a moderate temperature for an extended period of time. In single phase assays, the double-stranded duplexes may be separated from **single-stranded** nucleic acid by S.sub.1 nuclease digestion followed by precipitation of duplex molecules, or by selective binding to hydroxyapatite. In mixed. . .

DETD . . . signal strength of unknowns with that of the standards. It has previously been shown on Nytran slot blots with total **nucleic acid extracts** of a panel of 72 strains of 14 different bacteria that the signal strengths were comparable when hybridized with .sup.32. . .

DETD . . . live bacteria it is expected that probe cell count will generally be higher, since it detects the presence of total **nucleic acid isolated** from both viable and non-viable bacteria.

DETD . . . was stored at -20.degree. C. for several days. Upon thawing, the samples were treated with 1% W/V SDS and 1 **mg**/ml proteinase K. The total **nucleic acid** was **extracted** with two phenol-chloroform **extractions** and then precipitated with ethanol. The pellet was resuspended in TE (10 mM Tris, 1MM EDTA), heated for one minute. . .

DETD The total **nucleic acid** from a known number of actively growing cultured bacteria were **extracted** as above, then **nucleic acid** carefully **extracted**, serially diluted, slotted and subsequently probed with the same universal primer oligonucleotide. The resulting autoradiograph indicated the intensity of the. . .

DETD . . . bacteria including the following genera: Actinobacillus, Haemophilus, Bacteroides, Eikenella, Fusobacterium, Wolinella, Campylobacter, Escherichia, Peptostreptococcus, Streptococcus, Capnocytophaga, Selenomonas, Actinomyces and Fusobacterium. **Nucleic acids** from the different bacteria were **extracted** and slotted onto a Nytran filter. This filter was then probed with a kinased UP9A oligo in a 30% formamide,. . .

DETD . . . 20% N-hydroxymethyl-2-pyrrolidone, 10% N-dodecyl-2-pyrrolidone 50 mM Tris pH 7.6, 25 mM EDTA and 2% SDS(PLS) and containing 1 to 5 **mg** of 5 micron beads (silica, (Spherisorb) from Phase Sap, Deeside Ind., Queensferry, Clwyd, U.K.) onto which 1 to 2 micrograms. . .

DETD A pre-prepared solution composed of 0.2 **mg**/ml Proteinase K, 0.2% SDS in anaerobic growth media (brain heart infusion 30 g/l, soluble starch 10 g/l, gelatin 1 g/l. . .

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SUMM . . . of nucleotides having said hybridization capability. The probe can be free or contained within a vector sequence (e.g., plasmids or **Single Stranded** DNA).

SUMM The terms oligonucleotide or polynucleotide probes are meant to include both double stranded and **single stranded** DNA or RNA. The terms also refer to synthetically or recombinantly derived sequences essentially free of non-nucleic acid contamination.

DETD . . . RNA into a replication vector, such as pBR322, M13, or into a vector containing the SP6 promotor (e.g., generation of **single -stranded** RNA using SP6 RNA polymerase), and transformation of a bacterial host. The **DNA** probes can be **purified** from the host cell by lysis and **nucleic acid extraction**, treatment with selected restriction enzymes, and further **isolation** by gel electrophoresis.

DETD . . . DNA polymerase I, by tailing radioactive DNA bases to the 3' end of probes with terminal deoxynucleotidyl transferase, by treating **single-stranded** M13 plasmids having specific inserts with the Klenow fragment of DNA polymerase in the presence of radioactive deoxynucleotides, dNTP, by. . .

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